Spring 5-2017

Success of Artificial Insemination in Two Breeds of Maine Sheep is Not Hindered by Breed Differences

Dominic Barra
University of Maine

SUCCESS OF ARTIFICIAL INSEMINATION IN TWO BREEDS OF MAINE SHEEP IS NOT HINDERED BY BREED DIFFERENCES

by

Dominic Barra

A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Animal and Veterinary Sciences)

The Honors College

University of Maine

May 2017

Advisory Committee:

Dr. James Weber, Associate Professor and Animal and Veterinary Sciences, Advisor
David Marcinkowski, Associate Professor of Animal and Veterinary Sciences
Dr. Anne Lichtenwalner, Associate Professor of Cooperative Extension
Frank Dudish, Physics Lecturer
Samantha Jones, Professor of Art and Preceptor, Honors College

Abstract

The use of artificial insemination (AI) on sheep is an emerging field of research in the United States. Rates of AI success for frozen-thawed semen have been consistently lower than 30%; this makes long-term sperm storage for commercial use difficult. The method and semen extender used in this study were developed in Iceland and has consistently achieved success rates of 60% or higher. This study was designed to examine the effectiveness of this Icelandic method of AI in both Icelandic and East Friesian breeds of sheep. The results of our study show that East Friesian ewes achieved a success rate of 94.12% and the Icelandic ewes achieved a success rate of 50%. Although conclusions are limited by the size and design of the study, these results lead us to believe that there is no perceivable breed difference that would inhibit AI success in East Friesian sheep.

Acknowledgments

I would like to extend my thanks to Dr. James Weber for developing the initial research project and his help in making this project a possibility through his knowledge and expertise in the area of artificial insemination. I would also like to extend my gratitude towards my Honors thesis committee for giving me the motivation to push my knowledge and understanding of artificial insemination. My gratitude also extends to the Ewe-Maine sheep club for their assistance in monitoring ewes for heat and assisting with ewe handling.

Table of Contents

1. Introduction

1.1 Background

Recently the United States of America Customs regulations have become stricter on the importation of biological materials that may be vectors for disease [1]. The new regulations require a Center for Disease Control (CDC) certificate, which enforces a expensive testing on rams to prevent the spread of disease to the U.S.. This certification is leading many farmers in Iceland to stop exporting semen to America. The lack of semen being imported to some farms in Maine has caused a genetic standstill in most flocks since it is more difficult to select the best rams for mating due to distance. Our work on artificial insemination methods is important now due to the recent changes in Customs regulations leading to the inhibition of the importation of strong Icelandic sheep genes for U.S. flocks.

It is also important to examine how other breeds of sheep, such as the East Friesian breed, respond with the Icelandic process of artificial insemination (AI) as a method of improving genetics as well as preventing the spread of diseases from the transportation of rams to other farms for reproduction. The use of AI can improve both the welfare of the animal and advance the field of sheep genetics. The elimination of ram movement means that flocks are less likely to contract preventable diseases and parasites, improving animal welfare. The use of semen collection in breeding also means that more study can be conducted on genetic improvement in sheep flocks.

In previous studies conducted on UK breeds of sheep the use of fresh semen has yielded pregnancy rates of 70%, while frozen-thawed semen has yielded results closer to 30% [2,3,4]. These UK breeds included: Welsh Mountain draft ewes, Hardy Speckled Faced, Beulah, and Suffolk breeds of sheep. The exception to this general pregnancy rate of frozen-thawed semen is in Norway where they have achieved pregnancy rates in excess of 55% [5]. In these studies some researchers used egg yolk based extenders and some used skimmed milk based extenders.

In Iceland, pregnancy rates from chilled semen have been reported to be above 60% (personal communication, Thorstein Olafsson). This technique has only been shown to be useful on the Icelandic sheep breed due to a lack of implementation of this process with other breeds of sheep. The process includes collecting fresh semen from a ram on the same day as breeding to ensure the semen is still fresh and put in straws, but it is practice to slowly cool the semen down to 5 degrees Celsius using cold water in a thermos. This cooled semen can be transported across the country of Iceland without a decrease in fertility over time. To maintain temperature overnight it is advised to replace the old water with new 5 degree Celsius water [2]. The use of a skimmed milk semen extender with antibiotics may also contribute to the effectiveness of the process.

Iceland has also designed a system of distributing semen, which has yielded its own set of data indicating the effectiveness of AI in this breed, as well as the optimal timing to breed ewes after synchronization [6]. The data collected by Olafsson was only observed in the Icelandic breed of sheep [4]. Compared to other breeds of sheep who have been

extensively used in AI studies, there is a lack of knowledge specifically on how well East Friesian sheep react to AI. This lack of research means that it will be beneficial to begin examining this breed within this study.

The effectiveness of AI on sheep has not been studied extensively, and the United States has not been a major contributor to the study as a whole. A majority of studies have been done in countries where the major livestock raised is sheep, such as England, Ireland and Australia [5,2,3}. If more farmers in the US start raising sheep as livestock, it will be an important agricultural step to begin researching different methods of AI. The implication of artificial insemination in farms will improve biosecurity and improve the wellbeing of animals.

In a comprehensive paper on reproductive management of a flock of ewes [7], multiple techniques for synchronizing estrus using either progesterone or prostaglandin F2 alpha (PGF2a) are explained from a management standpoint. The effectiveness of PGF2a is reduced if ewes were less than 5 days post-estrus, which would suggest that progesterone is the better synchronizing technique if the ewes cycle is unknown at a given time. The only limitation to the use of progesterone is the ability for the delivery apparatus to maintain an effective dose of progesterone to prevent the ewe from returning to estrus after ovulation.

According to the textbook *Pathways to Pregnancy and Parturition* by PL Senger the cervix of a ewe is not a straight line that is typical with most species of animals [8].

There are random protrusions of rings that inhibit any straight path through the cervix, which prohibits the passing of a straight pipette. In order to directly deposit semen into the uterus, a laparoscope is needed to manipulate the straight pipette through the cervix. This procedure is costly for a farmer to perform on all the sheep on a commercial farm, and as such vaginal deposition of semen is the most common and cost effective method of insemination.

The number of ciliated cells within ovine cervix is higher than in other species of animals [9]. This increased number of ciliated cells can contribute to the reason why males depositing semen before the cervix still have high conception rates. It has also been found that during the luteal phase of a ewe's estrus cycle there are higher numbers of ciliated and mucosal cells within the cervix compared to other times during the ewes cycle [10]. While ewes are bred in the estrous phase, this increased number of cilia during the luteal phase illustrates how important cilia are in movement through the cervix.

It has also been found that there is no difference in cervical mucus composition between breeds of sheep that have an effect on inseminated semen motility [11]. This is notable as it means that AI effectiveness is not inhibited by the composition of cervical mucus. With this information one can assume that the formation of privileged pathways within the cervix occurs in a similar way despite the different cervical conformations that could be seen between multiple breeds. Within the cervix there are two types of cervical mucus, thick mucus and viscous mucus, which forms the privileged pathway. The thick

mucus is located towards the center of the cervix and pushes sperm that are not motile out of the cervix. The viscous mucus is located closer to the walls of the cervix and allows easier passage of sperm through the cervix, which is called a privileged pathway.

One belief about the reduction in pregnancy rate from frozen-thawed semen is that the seminal coating on the sperm is altered in the freezing process leading to lower rates of conception. A study by Ardon and Suarez on bull sperm [12] found that there was an increase in three different binders of sperm protein (BSP) coatings on sperm, which may be leading to the decrease in fertility as the sperm's coating is not the same as if it were left fresh. Since this study was conducted on bull sperm the exact interaction between the coated sperm and the cervical mucus may not be the exact mode of action, but it is possible that the extender can have similar results in sheep semen.

Sperm require an exogenous substrate to generate energy for motility through oxidative phosphorylation. A majority of this energy comes from sugars in seminal fluids, mainly fructose [13]. The job of the seminal fluid is to aid sperm in its movement through the cervix and allow them to survive until they reach the egg [14, 15], but survival of sperm does not last as long as many breeders would like, with un-extended semen in the right conditions can only last 1 to 2 hours at most. The downside to this process is that there is a buildup of toxic L-amino acid oxidase in the un-extended seminal fluid that can lead to damaging effects to the sperm [16]. As a result, an important component for AI is the semen extender, which acts as a buffer to prevent the toxic buildup in seminal fluid.

Another useful quality of the extender is to provide more nutrients for sperm energy production and motility as it serves as a source of sugars [13].

Extenders typically contain antibiotics in their mixture to prevent the spread to different farms common diseases whose pathogens are typically shed into genital secretions [17]. The most common antibiotics used in extenders are penicillin and streptomycin. Penicillin is an inhibitor of cell wall synthesis in bacteria. Its mode of action is to bind to a protein in a synthesized bacterial cell wall before the polymerization of the peptidoglycan layer [18]. This protein binding leads to the ultimate destruction of the bacterial cell due to the incomplete nature of its cell wall, and this effect can be achieved in both Gram positive and Gram negative bacteria. Streptomycin acts as a protein synthesis inhibitor by interfering with tRNA binding to the ribosomal subunit [19]. This interference prevents the completion of translation, which can be used for both Gram positive and negative bacteria. When these two antibiotics are used in conjunction the potential for disease transmission through seminal fluid is greatly decreased, and the chances of antibiotic-resistant bacteria surviving is also decreased.

In most commercial farms a method that results in early detection of pregnancy has advantages for farmers. The safest and most common method of early detection of pregnancy is the use of ultrasound. With this equipment farmers can immediately get pregnancy results on their flock and record this data before lambing season approaches, and this will allow them to prepare for an estimated number of lambs. Ultrasound data also allows a farmer to know which ewes have had reproductive performance in terms of

getting pregnant, number of lambs, and if these lambs are carried for the full term. In most countries sheep wool is only a byproduct of meat production, and lambs account for a portion of the sheep meat market. The earliest observable sign, based on ultrasonography, of fetal growth in the uterine horns of ewes appears at 21 days into gestation [20]. This knowledge can be applied to accurately determine the results of AI without requiring any non-pregnant ewes to miss lambing season. This means that AI can be performed early in the breeding season, and then ten days after AI a ram can be introduced and allowed to naturally breed with all open ewes to increase the total number of pregnancies. Ultrasonography is useful in this situation because it is possible for a person who is trained in ultrasonography to observe the different stages of pregnancy when there is a ten-day difference.

1.2 Hypothesis

Due to previous studies of artificial insemination (AI) in Icelandic sheep showing an average success rate of 70% with fresh semen using the Icelandic method of AI. It is hypothesized that the East Friesian breed of sheep will have a reduced effectiveness with the Icelandic method of AI.

2. Materials and Methods

The sheep used in this study are 16 Icelandic ewes found at the J. Franklin Witter Teaching & Research Center of Orono, ME, and 17 East Friesian ewes from Northern Exposure Farm of Holden, ME. The Icelandic ewes were inseminated with the semen from the Icelandic rams named Little Joe and Garpurson, while the East Friesian ewes were inseminated with semen of East Friesian rams named Monty and LBR. Semen from

both breeds was collected the day of breeding and the ewes were inseminated within 12 hours of their observed heat.

2.1 Synchronization of Ewes

The first part of our experiment used CIDR's (Controlled Internal Drug Release) that are impregnated with progesterone to synchronize the ewes (Eazi-Breed Sheep CIDR, Zoetis Animal Health, Kalamazoo, MI). The increased level of progesterone imitates the presence of a placenta during pregnancy. This prevents the ewes from going through their estrous cycle. It has been found that CIDR's are more aseptic and less likely to fall out when compared to the progesterone soaked sponges [21]. These were allowed to sit within the ewes for 12 days and primed synchronization of the entire flock [7]. On the eleventh day of CIDR implantation the ewes were given 1 mL of Estrumate, a synthetic prostaglandin $F_{2\alpha}$ analogue, to lyse the Corpus Luteum (Estrumate, Shering-Plough Animal Health, Summit, NJ). The CIDR was then removed, and ewes came into behavioral estrus (heat) within 2 to 3 days. This heat was skipped and the ewes went through another full estrous cycle until they came into heat a second time after CIDR removal, at which point the East Friesian ewes were bred. Due to time constraints with the Icelandic ewes, we were required to skip their second heat and wait for the third, approximately 30 days after CIDR removal. In an effort to preserve the synchronization of ewes so that they will be primed for insemination, we gave every Icelandic ewe a second dose of Estrumate.

We then inseminated two ewes artificially with chilled semen, before shifting to fresh semen with the rest of the ewes due to one ram being unsuitable for chilled semen. The

two ewes that were inseminated with the use of chilled semen were also inseminated with fresh semen at the next breeding session. The extended semen was deposited just caudal to the cervix in a way similar to the Icelandic technique. To ensure that all Icelandic ewes will provide lambs in the spring of 2017, ten days after our final AI we set the Icelandic ram Little Joe out to breed ewes that were not directly related to avoid inbreeding.

Heat watches were conducted 18-19 days after CIDR removal, the timing Olafsson in his personal communication with us indicated to be the most effective time to observe heats and achieve high pregnancy rates. For the East Friesian ewes this timetable was shown to be an accurate estimate for when to perform heat watches. However since the Icelandic ewes were not bred this cycle we only observed these heats and waited for the next cycle. This second heat cycle allowed for hands-on training for members of the Ewe-Maine Sheep Club to accurately observe signs of heat. Heats were determined through varying levels of behavioral estrous signs. The signs used as indication that a ewe should be bred within 12 hours were intense interest in the ram, increased vocalization and most importantly, tail wagging. The intense interest in a ram can be seen as a ewe staying close to the ram even when given the opportunity for food as well as aggressively pushing other ewes to try and get close to the ram. Increased vocalization was not the most accurate sign due to many ewes being normally vocal around people, but this should still be recorded in conjunction with the other signs. The most important sign we were watching for was the ewe aggressively wagging their tail around the ram, this sign is very evident and should be used as the sign that the ewe is ready to breed.

2.2 Ram Collection

While the ewes had the CIDR's implanted, the rams were halter trained to walk to where the ewes were held and "tease" them. This process took a while due to the rams being hesitant and resistant to working with people. To prevent unnecessary harm from the rams, only four well-trained people were allowed to halter the rams during this part of the project. While the CIDR was implanted this teasing had no effect on the ewes other than slight curiosity over the rams presence in their area. After the CIDR's were removed the rams were used to identify which ewes were in heat.

Before collection of the rams, a platform was constructed that elevated the animals to a height close to 3-3.5 feet high. This was made with wooden pallets and a ramp that was covered with a soft rubber mat. This mat served two purposes: increasing grip for the ascension up the ramp, as well as covering the open slots between the wooden boards of the pallet. The height of the platform was based on a subjective height that allows the animal to feel comfortable climbing and standing on it, while also allowing the collector the ability to work with the animal without bending over or lying on the ground. The platform was placed in an area where we could have one side of the animal against a barrier and where a ewe could be tied up in the front to prevent unsafe movement while the ram mounted her.

The procedure we followed was adopted from a paper written by Dyrmundsson et al. about the development of AI in Iceland [2]. First we lead the rams over to ewes that are located on the platform to stimulate them to mount a ewe. Then we used an artificial vagina (AV) filled with water at an internal temperature of 40-42 degrees Celsius to

stimulate the rams and collect their semen (Artificial Vagina for Small Ruminants, Catalog # 11320/0000, Minitube International, Fort Atkinson, WI). Diverting the penis away from the ewe and into the AV achieved this effect. To ensure the best possible collection from a ram, this process should be practiced with each ram before breeding collection until they are comfortable inseminating the AV. After each collection we did a sperm analysis and determined motility and concentration of the sperm. When it was determined that the ewes were ready to breed, we collected the males, performed the sperm analysis, and diluted the sperm concentration to a desired amount. We diluted the sperm in a skimmed milk extender and antibiotic mixture of 1:2.8 with a sperm concentration of 200 million sperm cells/mL for the ram Garpurson and 20 million sperm cells/mL for the ram Little Joe. We then loaded the semen into 0.5mL straws, and sealed them with a polyethylene powder that solidified to prevent leakage. We then cooled the straws from 30 degrees Celsius to 5 degrees Celsius over the course of 3-4 hours. The straws were then stored in 5-degree water in a Thermos (Thermos, Schaumburg, IL) brand vacuum flask until we inseminated the ewes.

To evaluate the quality of the sperm we made observations of semen volume and sperm motility. The semen volume was measured immediately after collection and then also after dilution. This was done using the collection cup at the end of the AV, which has measurement lines printed on it. Under a microscope we observed the motility of sperm in a drop of semen placed on a pre-warmed glass slide, looking for strong wave movements that are typical for sperm. To determine the concentration after collection we

used a hemocytometer, glass cover slide, and a known volume of counting buffer diluted sperm.

For the hemocytometer we had to follow a set procedure to ensure that our concentrations would be accurate and consistent for each count. We did this by first placing the cover slide over the middle of the hemocytometer. Then we loaded the hemocytometer by placing a drop of the killed semen onto the counting chamber of the hemocytometer to prevent the cover slide from moving off the middle of the hemocytometer. Figure 1 is an image of the counting chamber used in the hemocytometer to establish our sperm concentration; the counting frame is shown in the middle of the image where many grids are located. Capillary action from the grooves in the hemocytometer will move the semen into the middle grid, allowing us to count in an organized manner. To count, the hemocytometer was placed in the microscope and oriented for the middle of the hemocytometer to be located in the middle of the viewing space with the 40x lens. Then different magnifications were used to enhance the image of the middle grid until individual sperm could be observed. We then only counted sperm in the four corner cells of the middle square, and then the middle cell of the middle square for a total of five cells. To get the most accurate count of sperm, the fine magnification was adjusted to account for different layers of sperm on the hemocytometer, since it is not a flat slide. This value will be our number of sperm counted.

A hemocytometer is divided into 9 large squares, with the middle square divided into 25 medium sized squares. Each medium square side has a length of 0.20mm, with a total

area of 0.040mm^2 and a depth of 0.10mm. This gives each medium sized square a volume of 0.0040mm^3. In our evaluation we counted five of these squares giving us the total volume of 0.020mm^3, which equals 0.020μL since 1mm^3 is 1μL. This value is equivalent to 0.000020mL, which is our total volume in mL for the hemocytometer used in this study.

To account for the dilution of the sperm concentration from the extender and the counting buffer we used, we figured out dilution factors for each. To decide the dilution factor for our extender (EDF) we measured the original volume of semen from our collection in mL (V_O) and then added a volume of extender solution in mL (V_{Ext}). To account for the initial volume collected we divided the total volume by the initial volume ($\frac{V_O + V_{Ext}}{V_O} = EDF$) giving us a value without units. To determine the counting buffer dilution factor (CBDF) we took 1μL of the extender diluted volume (V_{Dil}) and added a volume of counting buffer in μL (V_{CB}) that would be easy to multiply our counted number of sperm by. We would then get our dilution factor the same way we calculated our extender dilution factor ($\frac{V_{Dil}+V_{CB}}{V_{Dil}} = CBDF$).

To calculate the original sperm concentration we used the equation:

Original concentration ($\frac{sperm\ cells}{mL}$) = $\frac{\#\ Sperm\ Counted}{Total\ V\ (mL)} \times EDF \times CBDF$

As an example we will demonstrate how we calculated the sperm concentration of Garpurson. The number we counted was 169, and our total volume is 0.000020mL. We added in 7mL of extender solution to our initial volume of 1mL, which gave us our EDF

value of 8. We took 1µL of the diluted semen and added in 99µL of counting buffer solution to give us a CBDF of 100. Putting these values into the equation above gave us an original concentration of $6.76x10^9$ sperm cells per mL.

2.3 Insemination

When we inseminated the ewes, we took the straws and placed one into our insemination gun. We cut the tip of the straw with the sealed powder off and covered the gun with a new protective sheath. We then captured a ewe and inseminated her by depositing the sperm in the vagina just caudal to the cervix. This was then repeated for each ewe, using a full straw per ewe, until we had inseminated all the ewes. After 30 days passed after the first AI, an ultrasound was used on the ewes to identify signs of pregnancy.

2.4 Data Collection

Data on the exact time ewes came into heat and were inseminated were recorded on paper and then transferred onto a Microsoft Excel spreadsheet. We also recorded if there were signs of fetal growth after the first ultrasound. The number of ultrasound-observed pregnancies was used to determine the effectiveness of AI in the two breeds of sheep. The timing after collection and cooling was also observed to extrapolate effects of time on fertility. Ram data was established after collection. This data includes sperm quality and the success of AI by each ram. Statistical analysis of data was done using tables and graphs to compare success of AI between the two breeds of sheep.

2.5 Figures

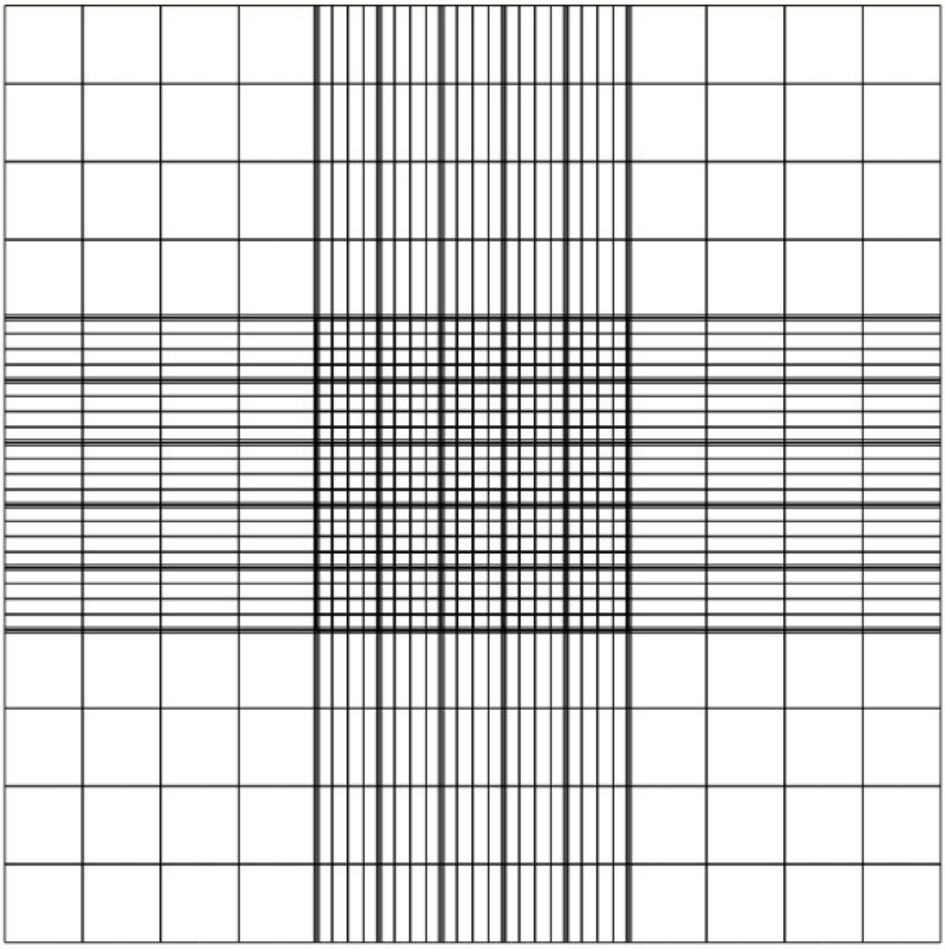

Figure 1: Hemocytometer counting chamber. Source: microbehunter.com/the-hemocytometer-counting-chamber/

3. Results

3.1 Analysis of Results

The sixteen Icelandic ewes used in this study had their CIDR's implanted on October 6th and removed on October 18th. The first and second cycles of heat that we skipped were observed on October 21st and November 5th, respectively. We observed signs of the third heat in the ewes as early as 5pm on November 15th; the last observed heats occurred around 5pm on November 16th. 2 ewes were inseminated in the morning of November 16th, 10 ewes inseminated in the afternoon of November 16th, and the final 4 ewes were

inseminated the morning of November 17th. On examining the ultrasound for embryo growth it was found that 8 of the artificially inseminated ewes had become pregnant from artificial insemination. The success rate of artificial insemination (AI) in this group of ewes was 50%.

The seventeen East Friesian ewes used in this study had their CIDR's implanted from October 18th to October 30th. The first signs of heat were observed on November 18th, and lasted until November 20th. Two ewes were bred on November 18th in the afternoon, ten ewes were bred in the morning of November 19th and another two in the afternoon of the same day, and the last three ewes were bred on November 20th. The ultrasound examination of the ewes showed that sixteen of the seventeen ewes had become pregnant from artificial insemination. The final success rate of AI in this group of ewes was 94.12%

Table 3 gives the data on each rams performance. The original concentration of each rams semen collection showed that Little Joe had a much lower sperm concentration than any of the other three rams in this study. Little Joe was only able to produce semen with a sperm concentration of 240 million sperm per milliliter of semen, while the other rams produced a concentration above 1 billion sperm per milliliter of semen. Because of Little Joe's low concentration, his diluted concentration in each straw was limited to 20 million per straw. Little Joe used a smaller dilution factor than the other rams, and even with this small dilution factor he had a low sperm concentration. The other three rams were able to provide straw concentrations of 200 million sperm/straw.

Figure 2 is a bar graph that illustrates the success rates of the different breeds with artificial insemination (AI). It is clear that the East Friesian group outperformed the Icelandic group with AI. It is also evident that the East Friesian breed performed higher than the expected 70% we see from fresh semen research by achieving a rate of 94.12%. The Icelandic sheep achieved a rate of 50%, which is similar to what was expected from chilled semen (60%), but 20% lower than the fresh semen rate (70%).

Figure 3 breaks up the success rate of artificial insemination to show individual ram success rates. The Icelandic ram Garpurson performed as expected for fresh semen AI with a success rate of 71%. The two East Friesian rams, Monty and LBR, achieved rates of 86% and 100%, respectively which is above the rates expected for fresh semen AI. Little Joe, the other Icelandic ram, only achieved a rate of 33%, which is approximately half of what is expected for chilled semen AI.

Figures 4 and 5 both illustrate the timing of ewes coming into heat, getting bred, as well as if that breeding session resulted in ewe pregnancy. According to Figure 3 the majority of East Friesian ewes came into heat on Day 19 Post-CIDR removal, with the afternoon of Day 19 being the most effective day for breeding to result in pregnancy. Figure 4 shows that the majority of Icelandic ewes came into heat on Day 30 post-CIDR removal with the afternoon of Day 30 also being the most effective day for breeding to result in pregnancy.

3.2 Tables and Figures

Ewe name:	CIDR placed	CIDR Removed	3rd Heat	AI Date	Preg. (AI) y/n	Garpurson or Joe AI	Preg. (Natural) y/n
Willow	10/6/16	10/18/16	16-Nov	16-Nov	Y	Joe	-
Fiddlehead	10/6/16	10/18/16	16-Nov	16-Nov	-	Joe	Y
Olive	10/6/16	10/18/16	16-Nov	17-Nov	-	Joe	Y
Petunia	10/6/16	10/18/16	16-Nov	16-Nov	Y	Joe	-
Lily	10/6/16	10/18/16	16-Nov	16-Nov	Y	Joe	-
Fleur	10/6/16	10/18/16	15-Nov	16-Nov	Y	Garperson	-
Elenor	10/6/16	10/18/16	16-Nov	17-Nov	Y	Garperson	-
Poinsetta	10/6/16	10/18/16	15-Nov	16-Nov	Y	Garperson	-
Clover	10/6/16	10/18/16	16-Nov	16-Nov	-	Joe	Y
Wasserbabe	10/6/16	10/18/16	16-Nov	16-Nov	Y	Garperson	-
Hawaii's Girl	10/6/16	10/18/16	16-Nov	16-Nov	-	Joe	Y
Dimon Girl	10/6/16	10/18/16	16-Nov	16-Nov	-	Joe	Y
Hawaii	10/6/16	10/18/16	16-Nov	17-Nov	-	Joe	Y
Cinnabelle	10/6/16	10/18/16	16-Nov	16-Nov	-	Garperson	-
Crazy Girl	10/6/16	10/18/16	16-Nov	17-Nov	-	Garperson	Y
Cinnabelle's Girl	10/6/16	10/18/16	16-Nov	16-Nov	Y	Garperson	-

Table 1: Raw Icelandic ewe data

Sheep #	CIDR placed	CIDR removed	2nd Heat Observed	AI Date	Pregnant y/n	Ram
Blue 7208	10/18/16	10/30/16	11/18/16	11/18/16	Y	Monty
Blue 8408	10/18/16	10/30/16	11/18/16	11/18/16	Y	Monty
Blue 8708	10/18/16	10/30/16	11/19/16	11/20/16	Y	LBR
Yellow 4109	10/18/16	10/30/16	11/19/16	11/19/16	Y	LBR
Green 20	10/18/16	10/30/16	11/18/16	11/19/16	N	Monty
Purple 22	10/18/16	10/30/16	11/18/16	11/18/16	Y	Monty
Red 39	10/18/16	10/30/16	11/18/16	11/19/16	Y	Monty
White 41	10/18/16	10/30/16	11/18/16	11/18/16	Y	Monty
Purple 66	10/18/16	10/30/16	11/19/16	11/19/16	Y	LBR
Orange 4	10/18/16	10/30/16	11/18/16	11/18/16	Y	LBR
Orange 7	10/18/16	10/30/16	11/18/16	11/18/16	Y	Monty
Orange 8	10/18/16	10/30/16	11/18/16	11/18/16	Y	LBR
Orange 13	10/18/16	10/30/16	11/17/16	11/18/16	Y	LBR
Orange 20	10/18/16	10/30/16	11/18/16	11/18/16	Y	LBR
Orange 30	10/18/16	10/30/16	11/18/16	11/18/16	Y	LBR

Orange 32	10/18/16	10/30/16	11/17/16	11/18/16	Y	LBR
Orange 39	10/18/16	10/30/16	11/17/16	11/18/16	Y	LBR

Table 2: Raw East Friesian Ewe Data

	Garpurson	Little Joe	LBR	Monty
Undiluted concentration	$6.7x10^9$/mL	$240x10^6$/mL	$3x10^9$/mL	$3x10^9$/mL
Straw concentration	$270x10^6$/straw	$20x10^6$/straw	$200x10^6$/straw	$200x10^6$ straw
#Bred AI	7	9	10	7
#Pregnant AI	5	3	10	6
% Success	71%	33%	100%	86%

Table 3: Raw ram performance data

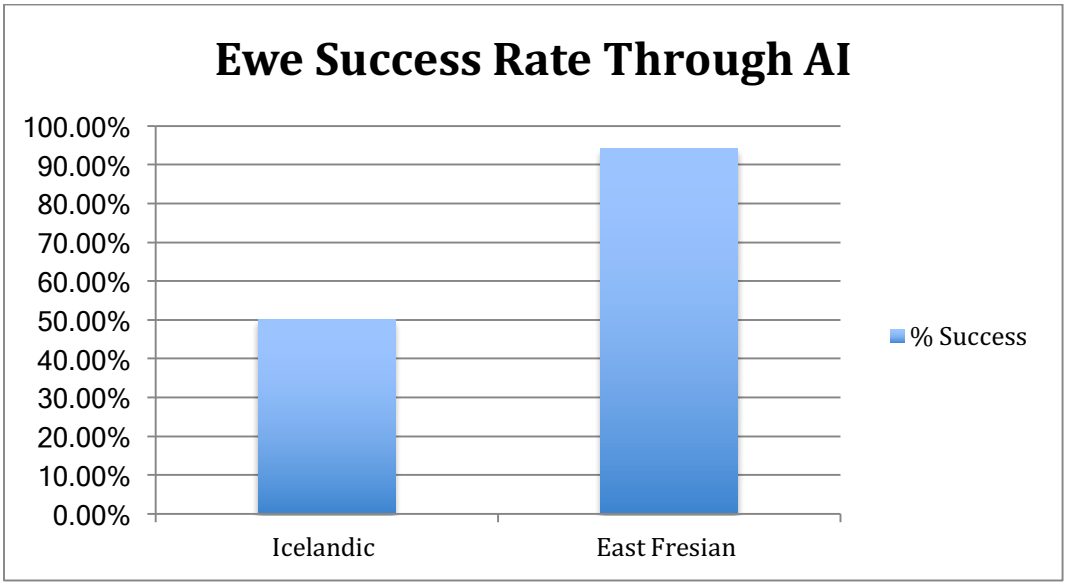

Figure 2: Comparison of Icelandic and East Friesian ewe Artificial Insemination pregnancy success

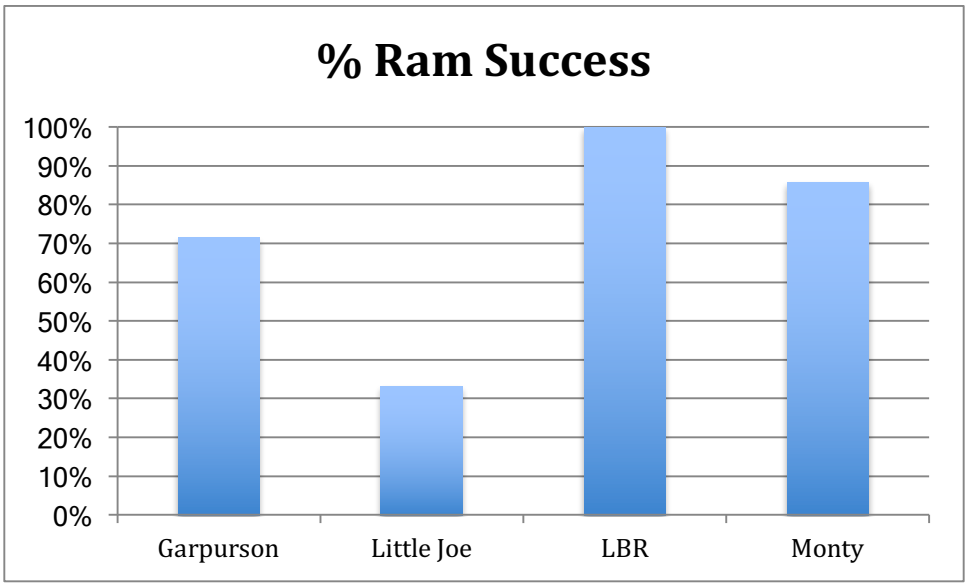

Figure 3: Percent Artificial Insemination success divided into rams.

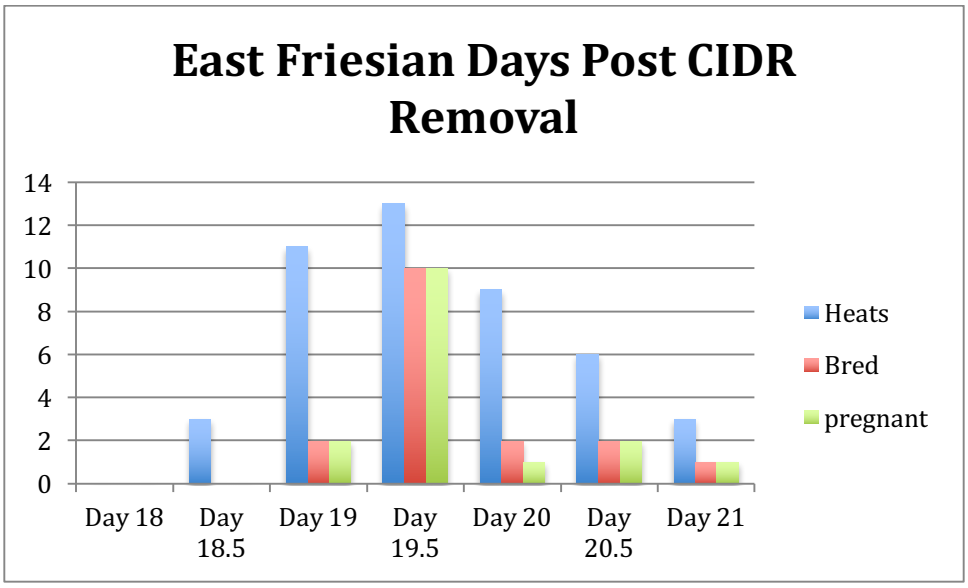

Figure 4: Comparison of days post CIDR removal between heats observed, ewes bred, and ewes pregnant in the East Friesian breed of sheep.

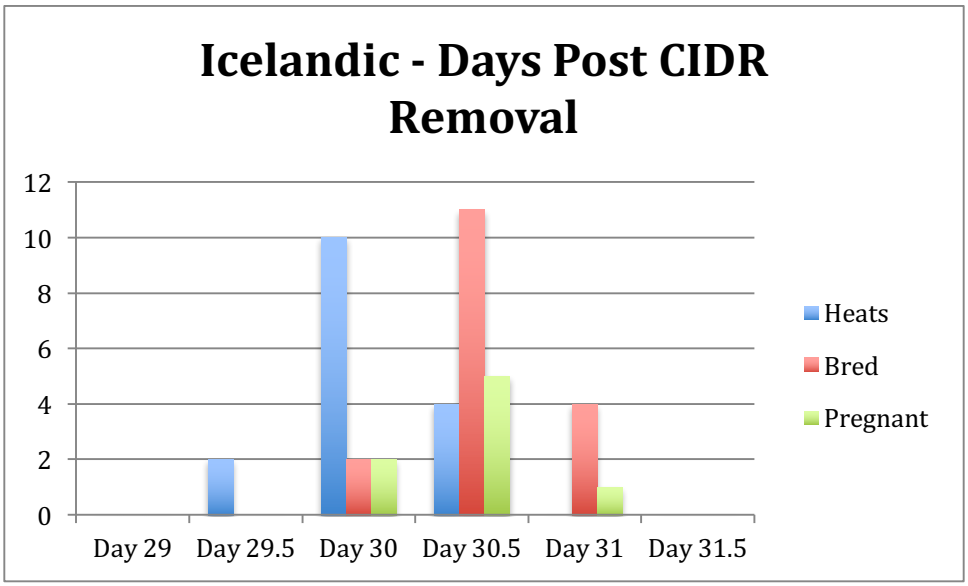

Figure 5: Comparison of days post CIDR removal between heats observed, ewes bred, and ewes pregnant in the Icelandic breed of sheep.

4. Discussion

The results of our study indicate that East Friesian sheep achieve high rates of pregnancy when bred with chilled semen delivered through AI. With 94.12% of ewes becoming pregnant through AI, the East Friesian group illustrated that the use of simple AI procedures with these sheep is a viable option for commercial farmers. The Icelandic group, however, performed poorer than expected with only 50% of ewes becoming pregnant through AI.

The results of this study are surprising as the procedure we followed for this study was optimized using the Icelandic breed of sheep. The fact that the East Friesians achieved a success rate above 90% is outstanding; this is a higher rate than the standard 70% most studies achieve with the use of fresh semen. The Icelandic group of sheep performed better than the typical rate of frozen-thawed insemination, 30%, but they achieved a

success rate lower than the average rate of fresh semen, 70%. The Icelandic breed did, however, approach the results for chilled semen AI, 60%.

The Icelandic group's rate of success is lower than we had desired, but was not unexpected for an initial study of this procedure. Due to the novelty of the process, the timing of insemination may not have been as accurate as what is achieved in Iceland. This study had a conflict of timing during the Icelandic group's second observed heat that prevented insemination at the optimal time outlined in the Icelandic AI procedures. Skipping this heat may have seen a decrease in synched estrous meaning that it is possible that it could have had an effect on the success rate of AI.

Another area that appears to have had a major effect on the Icelandic AI success was ram sperm concentration. Garpurson, one of the Icelandic rams, had a high sperm concentration both before, 6 billion sperm per mL of semen, and after, 270 million sperm per straw, dilution with the extender. This ram also achieved AI success in 71% of the ewes he had been bred with, which is the rate we would expect from fresh semen AI. Little Joe, the other Icelandic ram, had a low sperm concentration, with only 240 million sperm per mL of semen. When diluted with the extender we could only achieve a concentration of 20 million sperm per straw for insemination. Little Joe was also only able to achieve a 33% AI success rate, leading this researcher to believe that a ram's ability to produce high concentrations of sperm in its semen has a major effect on AI success.

It should also be noted that when collecting semen from both Icelandic rams, Little Joe gave us a noticeably smaller volume of semen from collection. This could be due to this ram not being comfortable with an artificial vagina (AV). Little Joe required a longer time than Garpurson to produce any amount of semen, and at this time it is unknown if what was collected was pre-ejaculate only or not. If it was pre-ejaculate then this means that the ram Little Joe can be just as fertile as the ram Garpurson, but less viable for AI usage. If farmers are going to be adopting AI into their farms, then all rams being used for semen collection should be trained and evaluated to observe if they will be viable options.

Both East Friesian rams achieved similar concentrations and achieved high AI success rates, above 70%. The concentration used in the East Friesian AI straws was roughly 200 million sperm/straw, a concentration similar to the ram Garpurson. This concentration seems to be effective for the insemination of ewes using the Icelandic method. By extension this concentration allows multiple straws to be made from a single collection, giving a viable means for a ram farm to make money from selling straws to farmers.

This study illustrates that the East Friesian breed of sheep does not have any discernable difference in cervical conformation that would inhibit the movement of extended sperm and AI success. This means that the protocol we used in this study can also be used on East Friesian sheep farms throughout the U.S.. Utilizing this protocol in commercial farms could provide more cost effective techniques for breeding and decrease the instances of disease that could be spread to a farm through the movement of rams. It will

also provide farmers with an effective tool for rapidly increasing their production through genomics and may lead the way for genomic testing and optimization as is used in the dairy industry.

The implication of this could be far-reaching, with great improvements in wool production and quality as well as milk production. If sheep farmers decide to adopt this method into their reproductive management then sheep species can see large increases in genetic potential within a short period of time compared to on-site ram breeding practices. The best method of introducing these AI procedures to farmers would be through veterinarians. Veterinarians have a responsibility to heal and protect animals from harm, and implementing these procedures will prevent the spread of diseases and parasites from one farm to another through the movement of rams. A veterinarian also has a good reputation with their clients that would allow them to inform farmers about these procedures and the benefits it can have for their protection and animal health.

It is possible that in previous studies where low success rates were achieved could have been due to the extender used to buffer semen. While researching, I took note of the fact that many studies used different extender mixes and many of these studies achieved a variable range of results. It is possible that the components of an extender can give variable buffering capacity to aid sperm survival, and as such cause lower success rates than the use of other semen extenders. More study should be done to determine the lengths of an extenders buffering capacity.

This study has provided an initial set of data that can be used as a basis for other studies assessing the effectiveness of AI in breeds of sheep. Due to our inability to follow the timing of breeding that was developed in Iceland, it would be beneficial for a follow up study to examine if there is an effect on success rate with different heat cycles. Another beneficial study would be examining how sheep from a variety of breeds react to the use of Icelandic AI methods. Finally a study of chilled semen AI effectiveness in Maine would extend the possibilities of AI throughout the state and even most of the country.

5. Conclusion

We have determined that our hypothesis has been rejected, and that East Friesian sheep farms are able to adopt the Icelandic method of AI without a decrease in productivity.

References

[1]. Import regulations for infectious biological agents, infectious substances, and vectors. U. S. Government Publishing Office 2016 November 18, 2016;2016(November 21):1.

[2]. Dyrmundsson OR, Jonmundsson JV, Olafsson T. The development of artificial insemination in sheep and goats in iceland. European Association for Animal Production 2007.

[3]. Haresign W, Khalid M, Scaramuzzi R. Development of trans-cervical AI in sheep. Project report to HCC.

[4]. Maxwell WM, Evans G, Mortimer ST, Gillan L, Gellatly ES, McPhie CA. Normal fertility in ewes after cervical insemination with frozen-thawed spermatozoa supplemented with seminal plasma. Reprod. Fertil. Dev. 1999;11:123-6.

[5]. Donovan A, Hanrahan JP, Lally T, Boland MP, Byrne GP, Duffy P, et al. AI for sheep using frozen-thawed semen. Armis 2001; 4047.

[6]. Donovan A, Hanrahan JP, Kummen E, Duffy P, Boland MP. Fertility in the ewe following cervical insemination with fresh or frozen-thawed semen at a natural or synchronised oestrus. Anim. Reprod. Sci. 2004;84:359-68.

[7]. Knights M, Hoehn T, March D, Lewis P, Pate J, Dixon A, et al. Reproductive management in the ewe flock by induction or synchronization of estrus. (In press).

[8]. Senger PL. Pathways to pregnancy and parturition. Third Edition. Redmon, OR 97756: Current Conceptions inc.; 2012.

[9]. Restall BJ, Lightfoot RJ. The distribution of ciliated cells in the cervix of the ewe. Aust. J. Biol. Sci 1970;23:1265.

[10]. Pathak D, Nagpal SK, Singh G. Scanning electron microscopic studies on uterus of sheep in follicular and luteal phases of estrous cycle. Indian J. Anim. Sci. 2008;78(12):1361; 1361,1364; 1364.

[11]. Richardson L, Hanrahan JP, O'Hara L, Donovan A, Fair S, O'Sullivan M, et al. Ewe breed differences in fertility after cervical AI with frozen-thawed semen and associated differences in sperm penetration and physicochemical properties of cervical mucus. Anim. Repro. Sci. 2011;129:37-43.

[12]. Ardon F, Suarez SS. Cryopreservation increases coating of bull sperm by seminal plasma binder of sperm proteins BSP1, BSP3, and BSP5. Reproduction 2013;146:111-7.

[13]. Rickard JP, Pini T, Soleilhavoup C, Cognie J, Bathgate R. Seminal plasma aids the survival and cervical transit of epididymal ram spermatozoa. Repro. 2014;11;148

[14]. Stefanov RG, Anev G, Abadjieva DV. Effect of different extenders and storage periods on motility and fertility of ram sperm. Macedonian Veterinary Review 2015;38(1):85; 85,89; 89.

[15]. Rekha A, Zohara BF, Bari FY, Alam MGS. Comparisons of commercial triladyl and locally manufactured extenders for the chilling of semen and their effects on pregnancy rates after transcervical AI in bangladeshi indigenous (ovis aries) sheep. Animal Reproduction 2016;13(4):735; 735,742; 742.

[16]. Martinus R, Molan P, Shannon P. Deleterious effect of seminal plasma in the cryopreservation of bovine spermatozoa. N. Z. J. Agric. Res. 1991;34(3):281; 281,285; 285.

[17]. Moustacas VS, Xavier MN, Carvalho-Junior CA, Costa EA, Henry M, Santos RL. Effect of extender supplementation with various antimicrobial agents on viability of brucella ovis and actinobacillus seminis in cryopreserved ovine semen. Theriogenology 2010;74(8):1476-81.
[18]. Liu Y, Breukink E. The membrane steps of bacterial cell wall synthesis as antibiotic targets. Antibiotics (Basel) 2016;5(3):28; 28.
[19]. Prabhu D, Vidhyavathi R, Jeyakanthan J. Computational identification of potent inhibitors for streptomycin 3"-adenylyltransferase of serratia marcescens. Microb. Pathog. 2017;103:94-106.
[20]. Jones AK, Gately RE, McFadden KK, Zinn SA, Govoni KE, Reed SA. Transabdominal ultrasound for detection of pregnancy, fetal and placental landmarks, and fetal age before day 45 of gestation in the sheep. Theriogenology 2016;85(5):939,45.e1.
[21]. Wheaton JE, Carlson K, Windels H, Johnston L. CIDR: A new progesterone-releasing intravaginal device for induction of estrus and cycle control in sheep and goats. Anim. Reprod. Sci. 10;33(1-4):127; 127,141; 141.

Author's Biography

Dominic Barra graduated the University of Maine in May 2017. In the Fall of 2017 Dominic will attend the University College Dublin School of Veterinary Medicine. He would like to pursue a specialty in small animal surgery after acquiring his degree. In the future Dominic wants to find a job at a small animal hospital until he can either find a job with a small town animal clinic or begin his own clinic in a small rural town.